Childhood asthma exacerbations and the Arg16 b2-receptor polymorphism: a meta-analysis stratified by treatment

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Childhood asthma exacerbations and the Arg16 β_2-receptor polymorphism: A meta-analysis stratified by treatment

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Background: The Gly-to-Arg substitution at the 16 position (rs1042713) in the β_2-adrenoceptor gene *(ADRB2)* is associated with enhanced downregulation and uncoupling of β_2-receptors.
Objectives: We sought to undertake a meta-analysis to test the hypothesis that there is an interaction between the A allele of rs1042713 (Arg16 amino acid) and long-acting β-agonist (LABA) exposure for asthma exacerbations in children.
Methods: Children with diagnosed asthma were recruited in 5 populations (BREATHE, Genes-Environments and Admixture in Latino Americans II, PACMAN, the Paediatric Asthma Gene Environment Study, and the Pharmacogenetics of Adrenal Suppression with Inhaled Steroid Study). A history of recent exacerbation and asthma treatment was determined from questionnaire data. DNA was extracted, and the Gly16Arg genotype was determined.
Results: Data from 4226 children of white Northern European and Latino origin were analyzed, and the odds ratio for exacerbation increased by 1.52 (95% CI, 1.17-1.99; $P = .0021$) for each copy of the A allele among the 637 children treated with inhaled corticosteroids (ICSs) plus LABAs but not for treatment with ICSs alone (n = 1758) or ICSs plus leukotriene receptor antagonist (LTRAs; n = 354) or ICSs plus LABAs plus LTRAs (n = 569).
Conclusions: The use of a LABA but not an LTRA as an "add-on controller" is associated with increased risk of asthma exacerbation in children carrying 1 or 2 A alleles at rs1042713. Prospective genotype-stratified clinical trials are now required to explore the potential role of rs1042713 genotyping for personalized asthma therapy in children. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Adrenergic receptors, asthma, child, disease exacerbation, therapeutics*

Asthma is a common condition in children in which there is heterogeneity in response to treatment with inhaled corticosteroids (ICSs), long-acting β-agonists (LABAs), and leukotriene receptor antagonists (LTRAs).[1,2] Some of this heterogeneity might reflect genetic variations within the population, and variants in the β_2-adrenoceptor gene *(ADRB2)* have been associated with increased risk for symptoms.[3-5] Of particular interest is the single nucleotide polymorphism (SNP) rs1042713, a Gly-to-Arg amino acid substitution at position 16 of the *ADRB2* gene that has been associated with differences in pulmonary function

Abbreviations used
ADRB2: β_2-Adrenoceptor gene
GALA II: Genes-Environments and Admixture in Latino Americans
HWE: Hardy-Weinberg equilibrium
ICS: Inhaled corticosteroid
LABA: Long-acting β-agonist
LTRA: Leukotriene receptor antagonist
OR: Odds ratio
PAGES: Paediatric Asthma Gene Environment Study
PASS: Pharmacogenetics of Adrenal Suppression with Inhaled Steroid Study
SABA: Short-acting β-agonist
SNP: Single nucleotide polymorphism

responsiveness to short-acting β-agonists (SABAs) in children.[6-9] The underlying mechanism of enhanced downregulation and uncoupling of β_2-receptors is thought to reflect an altered response to SABAs and LABAs.

Although the SNP rs1042713 appears to alter physiologic and clinical responses to SABAs and LABAs in pediatric populations, the clinical relevance of this association remains unclear. In 2 clinical trials there was no evidence for an association between the A allele of rs1042713 (Arg16 amino acid) and increased symptom scores.[1,7] There is inconsistent evidence from observational studies that this SNP might be relevant to exacerbations. In children the homozygous G/G genotype of rs1042713 has been linked to increased risk for hospitalization,[10] reduced bronchodilator response to SABAs,[9] prolonged stay in the hospital,[11] and intensive care unit stay[12] after presentation with acute asthma, whereas the heterozygous genotype of rs1042713 has been linked to increased risk for intubation for acute asthma.[13] Two other groups have observed associations between the A/A genotype of rs1042713 and increased exacerbations among those treated with LABAs,[3,4] but this was not confirmed in a third population.[14] These studies have also observed increased exacerbation risk[3] and poorer asthma control[4] among those children homozygous for A/A for the SNP rs1042713 receiving ICSs (but not LABAs). In one study[3] there was evidence that concomitant LTRA treatment might negate any increased risk for exacerbation associated with LABA treatment, whereas those children who are homozygous for Arg16 had better asthma outcomes when treated with LTRAs rather than LABAs in addition to ICSs.[15] Prospective studies undertaken in adult populations have found no evidence for LABA treatment being associated with adverse outcomes when added to ICS treatment.[16-18]

To better understand the interactions between the SNP rs1042713 of *ADRB2* and asthma treatment, we undertook a meta-analysis of results from 5 previously described populations.[19] Our hypothesis was that there is an interaction between the A allele of rs1042713 (Arg16 amino acid) and treatment with LABAs but not LTRAs for asthma exacerbation risk and that this risk might be further increased by exposure to daily SABAs.

METHODS

Study design

Asthmatic children were recruited to 5 cross-sectional studies (BREATHE, Genes-Environments and Admixture in Latino Americans II [GALA II], the Paediatric Asthma Gene Environment Study [PAGES], PACMAN, and PASS). The BREATHE and PAGES populations were recruited from primary and secondary care in Scotland, the PACMAN population was recruited from children attending community pharmacies in The Netherlands, GALA II recruited children in the United States and Puerto Rico who had 4 Latino grandparents, and PASS recruited children with asthma who had adrenal suppression testing in 25 hospitals across the United Kingdom. Further details of the study population's recruitment are presented in the Methods section in this article's Online Repository at www.jacionline.org. DNA was extracted from saliva or blood, and the genotypes for rs1042713 were determined. The primary outcome was asthma exacerbation (with reference to 6 months in BREATHE, PAGES, and PASS and 12 months in GALA II and PACMAN). Asthma treatment was categorized as follows: (1) as-required SABA but no preventer treatment, (2) ICS monotherapy plus as-required SABA, (3) ICS and LABA plus as required SABA, (4) ICS and LTRA plus as required SABA, and (5) ICS, LABA and LTRA plus as required SABA. As defined previously,[5] use of as required SABAs was categorized as at least once daily or less frequently. Approval was obtained from medical research ethics committees from each institute before recruitment. All participants provided verbal assent, and parents or participants provided written consent, as appropriate.

Definitions of exacerbation

For BREATHE and PAGES, the definition of exacerbation was at least 1 of the following in the previous 6 months in the context of asthma symptoms: hospital admission, course of oral steroids, or absence from school. For GALA II, an exacerbation was defined as at least 1 of the following during the previous 12 months: oral corticosteroid rescue treatment, hospitalization, or need to seek emergency asthma care. For PACMAN, an exacerbation was defined as an asthma-related visit to the emergency department, prescription of a course of oral steroids in the past 12 months, or both. The definition of exacerbation for PASS was at least 1 course of rescue oral steroids in the previous 6 months.

DNA collection, extraction, and analysis

For BREATHE, PACMAN, and PAGES, saliva was collected in commercially available pots (Oragene; DNA Genotek, Ontario, Canada), DNA was prepared with the Qiagen DNeasy 96 Kit (Qiagen, Hilden, Germany), and genotypes were determined in the Dundee laboratory by using TaqMan-based allelic discrimination assays on an ABI 7700 Sequence Detection System (Applied Biosystems, Foster City, Calif), as described previously.[3] For GALA II, DNA was extracted from whole blood, and the Axiom LAT1 array (World Array 4; Affymetrix, Santa Clara, Calif) was used to determine genome-wide genotype data, as described elsewhere.[20] For PASS, the Illumina Human OmniExpressExome-8 v1.0 chip (Illumina, San Diego, Calif) was used for genotyping.

Statistical analysis

The primary outcome was recent exacerbation, and this was related to genotype in logistic models. An additive model[3] was used (ie, a gene/dosage effect for the A allele [Arg16 amino acid]), which adjusted for confounders (ie, sex, age, and second-hand smoke exposure[3]). Each population was stratified by treatment, and risk for exacerbation per genotype was calculated in each treatment group. Daily SABA use was recorded for BREATHE, PACMAN, and PAGES, and here an interaction was sought for SABA treatment × genotype. Regression analyses in GALA II included the same covariates as in the other studies, but additionally, we included estimates of global African and Native American genetic ancestry to avoid confusion because of population stratification. Standard statistical software was used (SPSS version 22.0.0.1; SPSS, Chicago, Ill). The meta-analysis of data from the 5 populations was performed by using a fixed-effect (inverse variance–weighted) model in which the effect size estimates, β-coefficients, are weighted by their estimated SEs by using GWAMA software.[21] We estimated the power of the study to detect associations with

TABLE I. Comparison of details of children in each of the 5 study populations

	BREATHE (n = 1210)	GALA II (n = 1171)	PACMAN (n = 760)	PAGES (n = 695)	PASS (n = 390)
Male sex, % (no.)	60 (725)	58 (676)	63 (478)	57 (399)	56 (172)
Exposed to tobacco smoke at home, % (no.)	35 (424)	21 (242)	14 (108)	21 (146)	36 (108)
Mean age, y (SD)	9.7 (3.8)	11.9 (2.7)	8.7 (2.3)	9.8 (3.7)	11.1 (4.0)
Recent exacerbation, % (no.)	44 (536)	65 (763)	10 (76)	47 (323)	75 (295)
Ethnicity, %					
White	No data	0	90* (681/753)	93† (358/384)	99 (388/390)
Hispanic		100	0.4 (3/753)	0	0
African		0	1 (8/753)	0	0
Other (including mixed)		0	8.6 (61/753)	7 (26/384)	1 (2/390)
Minor allele frequency¶	0.37	0.45	0.41	0.37	0.38
rs1042713 genotype, % (no.)					
A/A (Arg/Arg)	15 (175)	20 (234)	15 (115)	14 (96)	16 (61)
A/G (Arg/Gly)	43 (515)	49 (579)	51 (388)	46 (321)	43 (169)
G/G (Gly/Gly)	43 (520)	31 (358)	34 (257)	40 (278)	41 (160)
Treatment group, % (no.)					
SABA alone	18 (218)	42 (490)	10 (73)	7 (51)	0
ICS	58 (698)	24 (283)	63 (476)	40 (273)	7 (28)
ICS plus LABA	11 (138)	10 (122)	19 (147)	19 (134)	33 (96)
ICS plus LTRA	5 (65)	15 (177)	3 (23)	9 (65)	8 (24)
ICS plus LABA plus LTRA	8 (91)	9 (99)	5 (41)	24 (169)	59 (230)
With daily SABA dosing, % (no.)	21 (250)	‡	49 (364)	30 (209)	‡

*Dutch, Moroccan, and Turkish ethnicities were considered white in PACMAN.
†Ethnicity data were not available for all participants in PAGES.
‡Daily SABA status was not determined in GALA II and PASS.
¶Minor allele frequency = Frequency of minor allele homozygous genotype + (Frequency of heterozygous genotype)/2.

TABLE II. OR for exacerbation per copy of the A allele (Arg16 amino acid)

	OR (95% CI) for exacerbation per A allele (referenced to none)						
Treatment group	BREATHE	GALA II	PACMAN	PAGES	PASS	Results from all cohorts combined	Results for all cohorts except GALA II
SABA alone	0.87 (0.54-1.40), n = 218	1.08 (0.81-1.43), n = 490	1.07 (0.06-20.2), n = 73	0.71 (0.18-2.80), n = 51	*	1.01 (0.79-1.28), n = 832, P = .95	0.85 (0.55-1.33), n = 342, P = .49
ICS alone	1.15 (0.92-1.43), n = 698	1.12 (0.78-1.62), n = 283	0.83 (0.53-1.31), n = 476	1.17 (0.80-1.71), n = 273	4.81 (0.79-29.33), n = 28	1.11 (0.95-1.31), n = 1758, P = .18	1.11 (0.94-1.33), n = 1475, P = .22
ICS+LABA	1.52 (0.92-2.50), n = 138	2.07 (1.03-4.16), n = 122	2.54 (1.06-6.06), n = 147	1.29 (0.76-2.19), n = 134	1.21 (0.68-2.14), n = 96	1.52 (1.17-1.99), n = 637, P = .0021	1.44 (1.08-1.93), n = 515, P = .01
ICS+LTRA	1.86 (0.85-4.08), n = 65	1.26 (0.79-2.02), n = 177	2.10 (0.43-10.2), n = 23	0.69 (0.34-1.39), n = 65	0.31 (0.08-1.18), n = 24	1.11 (0.80-1.55), n = 354, P = .52	0.98 (0.61-1.56), n = 177, P = .93
ICS+LABA +LTRA	1.03 (0.54-1.96), n = 91	0.93 (0.36-2.39), n = 99	0.23 (0.04-1.46), n = 41	0.87 (0.52-1.45), n = 169	1.02 (0.70-1.48), n = 169	0.94 (0.73-1.22), n = 569, P = .65	0.95 (0.72-1.24), n = 470, P = .68

Results are from logistic regression models that adjusted for sex, age, and exposure to secondhand smoke at home.
*There were no children in PASS receiving SABAs alone.

exacerbations according to the methodology of Purcell et al.[22] Our power calculations provide the maximal power we could obtain from the meta-analysis of the cohorts at a significance level of 5%. Odds ratios (ORs) of 1.2, 1.5, and 3 were selected based on initial results from the BREATHE population. With the exception of the ICS+LTRA treatment group, all strata were sufficiently powered to detect an OR of 1.5 or greater (see Table E1 in this article's Online Repository at www.jacionline.org). Forest plots were generated with the package *rmeta* for R software. A P value of less than .05 was assumed to be significant.

RESULTS

Study subjects

Genotype, treatment, and exacerbation data were available for 4226 children, including 1210 from the BREATHE, 1171 from GALA II, 760 from PACMAN, 695 from PAGES, and 390 from PASS (Table I). The Gly16Arg polymorphism was in Hardy-Weinberg equilibrium (HWE) for all cohorts with the exception of BREATHE (exact test P = .012) considered as a whole, but

TABLE III. The proportion (percentage) of children with exacerbations stratified by treatment class and SNP rs1042713

	BREATHE			GALA II		
Treatment group	**AA**	**AG**	**GG**	**AA**	**AG**	**GG**
SABA alone	7/29 (24%)	28/101 (28%)	25/88 (28%)	57/92 (62%)	150/241 (62%)	88/157 (56%)
ICS alone	52/98 (53%)	121/296 (41%)	126/304 (41%)	43/62 (69%)	93/151 (62%)	46/70 (66%)
ICS+LABA	18/22 (82%)	25/55 (46%)	33/61 (54%)	25/30 (83%)	43/56 (77%)	23/36 (64%)
ICS+LTRA	8/11 (73%)	18/26 (69%)	15/28 (54%)	22/34 (65%)	59/81 (73%)	39/62 (63%)
ICS+LABA+LTRA	11/15 (73%)	23/37 (62%)	26/39 (67%)	13/16 (81%)	37/50 (74%)	25/33 (76%)

it was in HWE in the group of children without exacerbations ($P = .624$). The minor allele frequency for GALA II was higher when compared with that of the 3 United Kingdom cohorts (0.45 vs 0.37, $P = 1 \times 10^{-10}$) and intermediate for the PACMAN population. Regardless of treatment, across the 5 populations, the additive model found an increased risk for exacerbation for each copy of the A allele amounting to 1.11 (95% CI, 1.01-1.22; $P = .035$, n = 4226; see Table E2 in this article's Online Repository at www.jacionline.org).

Risk of exacerbation across maintenance treatment groups

The OR for exacerbation was 1.52 (95% CI, 1.17-1.99) for each copy of the A allele among the 637 children treated with an ICS plus a LABA (Table II). The risk for exacerbation was not increased among other treatment groups (Table II). Table III presents the proportion of children with exacerbations stratified by population, treatment, and genotype. The analysis for children treated with an ICS plus a LABA had greater than 90% power to detect an association with increased risk for exacerbation at a significance level of 5% (see Table E1). An analysis of local African ancestry at the Gly16Arg locus was undertaken in the GALA II population to examine whether the number of chromosomes indicative of African ancestry at this locus was associated with increased exacerbations. There was no association of local African ancestry with exacerbations in GALA II in the overall population (OR, 1.17; 95% CI, 0.89-1.53; $P = .270$) or in the group of patients treated with an ICS plus a LABA (OR, 1.78; 95% CI, 0.58-5.49; $P = .316$).

Risk of exacerbation in relation to SABA use

Among the 822 children in receipt of daily SABAs (including 56 who were not receiving ICSs, LABAs, or LTRAs), there was no evidence of increased risk in the additive model (OR, 1.01; 95% CI, 0.79-1.31; see Table E3 in this article's Online Repository at www.jacionline.org). Among those children in receipt of an ICS plus a LABA, there was no evidence of any additional increased risk in relation to each A allele for exacerbations among those receiving daily SABAs (see Table E4 in this article's Online Repository at www.jacionline.org).

Asthma control scores and Arg16 homozygous genotype

The risk for poorly controlled asthma (as evidence by an Asthma Control Questionnaire 6 score >1.5) was increased among A/A homozygotes prescribed ICSs only within the PACMAN cohort (OR, 2.15).[4] Within the PAGES population, 63% (282/446) had poorly controlled asthma (as evidenced by a Children's Asthma Control Test score <20), and there was no increase in risk for poor control for A/A homozygotes among any of the treatment groups.

DISCUSSION

Genetic epidemiology is complicated by inconsistent findings between populations. Therefore replication of findings across different populations is crucial to generalizing results.[23] Associations between SNP rs1042713 and LABA and SABA treatment have been previously reported in evaluations of the first 546 children recruited to BREATHE[3] and the first 597 recruited to PACMAN[4] (data from 1210 and 760 included in the present report, respectively). However, the results of other studies in adults were in apparent conflict with the above observations. This meant that before this study, the important clinical question of whether there is a need to progress to further randomized controlled trials assessing benefit with testing for SNP rs1042713 in the clinical setting had not been resolved. This study combined data from 5 cohorts of children with asthma from white European and Hispanic/Latino populations to explore interactions between exposures to different asthma medications and the SNP rs1042713 for risk of asthma exacerbation.

We analyzed data from 4226 children and drew 3 conclusions. First, among children exposed to ICS plus LABA treatment as dual-combination therapy, there was a 52% increased risk for exacerbation for each copy of the A allele. Second, the interaction between the A allele and exposure to LABAs was not present when LTRA treatment was also coprescribed as triple therapy. Third, there was no evidence that daily SABA use in addition to ICS plus LABA treatment was associated with any increased further risk for exacerbation among children carrying at least one A allele. The combined incidence of the A/G heterozygous and A/A homozygous genotype is approximately 60%, and these observations implicate the SNP rs1042713 as an important factor in the well-recognized heterogeneity of treatment response in children with asthma.[1,2] This study has established the need for further prospective clinical trials in which treatment is stratified by genotype to move these observations into clinical practice to evaluate a more personalized approach to treatment of children with poorly controlled asthma despite treatment with ICSs.

We observed heterogeneity between populations for the relationship between SNP rs1042713 and treatment with an ICS plus LABA and risk for exacerbation, with the risk being highest in GALA II and lowest in PASS. Although this study was not designed to explain the variability between populations, there was

TABLE III. *(Continued)*

PACMAN			PAGES			PASS		
AA	AG	GG	AA	AG	GG	AA	AG	GG
0/10 (0%)	2/42 (5%)	0/21 (0%)	0/8 (0%)	5/23 (22%)	4/20 (20%)	0	0	0
6/76 (8%)	24/243 (10%)	19/157 (12%)	15/38 (40%)	33/122 (27%)	35/113 (31%)	1/2 (50%)	7/15 (47%)	2/11 (18%)
6/20 (30%)	3/73 (4%)	4/54 (7%)	11/18 (61%)	36/64 (56%)	25/52 (48%)	8/17 (47%)	25/41 (61%)	16/38 (42%)
1/5 (20%)	3/9 (33%)	1/0 (11%)	5/11 (46%)	16/27 (59%)	17/27 (63%)	2/5 (40%)	2/7 (29%)	10/12 (83%)
0/4 (0%)	3/21 (14%)	4/16 (25%)	14/21 (67%)	58/82 (71%)	47/66 (71%)	17/35 (49%)	58/101 (57%)	48/94 (51%)

no obvious association between the effect size for exacerbation risk associated with the A allele and characteristics of the 5 populations; for example, the children in GALA II and PASS were comparable in terms of age, sex distribution, and exacerbation rate. More children in PASS were in receipt of an ICS plus LABA compared with those in GALA II, but the hypothesis that exacerbation risk attributable to the A allele is lower for populations in which LABA treatment is more prevalent is not supported by observations in the PACMAN and PAGES populations, where 19% in each received LABAs, but the exacerbation risk associated with ICS plus LABA use was 2.54 and 1.29, respectively. The heterogeneity between populations and within populations[1,2] might provide potential insight into the pharmacogenetic mechanism or mechanisms but also highlights the need for stratified treatment in childhood asthma.

The minor allele frequency was substantially higher for children in the GALA II population compared with those in the 3 United Kingdom populations, and as suggested by previous work,[18,24] we explored the possibility that the increased exacerbation rate associated with the Arg16 allele in LABA-treated GALA II subjects reflected the African ancestry associated with this allele. In our adjustment for measures of ancestry for the analyses of the Gly16Arg locus within the GALA II population, we did not find significant evidence that African ancestry was relevant to the positive correlation between minor allele frequency and prevalence of exacerbation; however, our analysis was underpowered, and the 2-fold increase in risk detected might have been significant had our sample size been larger. Our study was not designed to explore how ethnic differences might be relevant to the pharmacogenetics or treatment response to LABAs, and unfortunately, there were insufficient numbers of children with African ancestry in the cohorts other than GALA II to further explore this intriguing hypothesis, which merits focused research in the future.

The pharmacogenetics of LABAs and SABAs are notable for the contrasting effects seen for the Gly16Arg locus on acute versus chronic SABAs. There has been considerable consistency in the observed effects of Gly16Arg on acute SABA response, with many studies showing a similar direction of effect on bronchodilation (favoring Arg16).[6,7,11,25,26] A seemingly opposite effect (favoring Gly16) was seen for chronic SABA exposure and lung function and asthma control in the Beta Agonists in Mild Asthma[27] and Beta-Adrenergic Response by Genotype[28] studies and another study by Taylor et al.[29] The focus of the present study was LABA therapy, but we found no evidence for either daily SABA use or the combination of a daily SABA plus LABA being linked to increased exacerbation risk for children carrying the Arg16 allele. One interpretation of our findings is that the LABA caused effective adrenoceptor blockade, and the "adverse" effect of LABA treatment had subsumed any "benefit" of acute SABA treatment for individuals carrying an Arg16 allele.

Although we find evidence here for response to LABA therapy to be modified by the Gly16Arg locus in children, for adults, this locus appears to have no effect on response to LABA therapy based on large retrospective analyses and prospective genotype-stratified clinical trials by Bleecker et al[16] and Weschler et al.[18] There is evidence that the Gly16 allele might be associated with a bronchoprotective effect in association with LABA treatment.[18,30] The present study was not designed to explore the apparent inconsistency between observations in adults and children, but differences in response of children and adults to LABAs are well recognized. The addition of a LABA to ICS treatment in adults with poorly controlled asthma is accepted to be superior to alternative treatments,[31,32] but in children the evidence is that LABAs are no more effective than addition of an LTRA or an increase in ICS dose.[1,33]

In the literature there are apparently conflicting associations reported between variants of the SNP rs1042713 and response to treatment.[3-6,9,11] One explanation for this heterogeneity is that the earlier findings are based on relatively small single populations (ie, which number less than 1000) and that a meta-analysis addresses the potential for false-positive findings and/or associations, which are idiosyncratic for one population. This meta-analysis confirms previously reported increases in the risk for exacerbations among Arg16 homozygotes in receipt of LABA treatment.[3,4] The present study did not replicate previously reported associations between the homozygous genotype A/A and treatment with ICSs alone and increased risk for exacerbation[3] or poor asthma control,[4] suggesting the possibility of false-positive findings. The magnitude of risk for exacerbation associated with ICS and LABA treatment and the A allele reported in the first 546 children recruited to the BREATHE cohort[3] is slightly reduced in the larger population (2.1 vs 1.5) but remains significant across all 5 populations. The 5 populations included were heterogeneous for asthma outcomes, and the results could be generalized to Western European and Hispanic/Latino populations, but given the potential for different associations between rs1042713 and asthma treatment response between different ethnic groups,[26] our results might not be relevant to all populations.

In our analysis we explored the possible additive effect of treatment with daily SABAs and LABAs for exacerbations, and we have previously reported that either treatment is associated with increased exacerbations for the BREATHE population among Arg/Arg homozygotes.[5] When data were pooled, there was no apparent additive effect of SABAs on LABA use for exacerbation risk for children with 1 or 2 Arg alleles. These results should be treated with caution because, even with a relatively large population, such as we present here, there were relatively

few children with ICS plus LABA and daily SABA exposure, and the analysis was probably underpowered. There might be a modest additive effect, which we were not able to detect.

The mechanism or mechanisms underlying the association between the Arg16 allele and increased exacerbation in the context of LABA treatment are thought to be mediated by enhanced agonist-induced downregulation and receptor uncoupling, resulting in subsensitivity of response.[34,35] Our novel finding of no increased risk for exacerbation among A/A homozygotes in receipt of ICSs, LABAs, and LTRAs suggests that factors other than *ADRB2* downregulation are active because LABA exposure in this group of children might be expected to downregulate *ADRB2*. It is likely that LTRAs merely confer an additional anti-inflammatory effect in subjects exposed to LABAs, such that in genetically susceptible patients it might be seen as a salutary effect by counteracting the response to LABAs. Where the LABA effect is negated (ie, in Arg/Arg), the additive effect of LTRAs will be more evident compared with a setting in which the LABA effect is more pronounced (ie, Gly/Gly), and the additive effect of LTRA will be less evident. Indeed, in one study using AMP challenge as the primary outcome, there was better protection with ICS+LABA+LTRA as triple therapy compared with dual therapy with ICS+LABA, which was also mirrored by effects on exhaled nitric oxide levels and blood eosinophil counts, suggesting that the apparent counteracting role of LTRAs might arise from the additional anti-inflammatory effects of LTRAs.[36]

This study has a number of limitations that should be considered when these results are interpreted. First, the associations described here do not imply causation, but the findings are consistent with the results of a small genotype-stratified randomized controlled trial, which found favorable outcomes among A/A homozygotes taking LTRAs compared with LABAs over a period of 12 months when used as add-on therapy to ICSs.[15]

Second, although we are able to be conclusive as to the nature of the relationship between exposure to LABAs and A/A homozygous genotype and exacerbation, we cannot exclude the possibility that there might be a small additive relationship between SABAs and LABAs for exacerbation. The relationship between LABAs, SABAs, and exacerbations will always be a challenge to study because frequent SABA treatment is an indication for LABA therapy, but our findings suggest that the magnitude of association with LABAs is greater than with SABAs, which perhaps is not surprising given the potential effect of more prolonged receptor occupancy conferred by LABAs than SABAs, especially in genetically susceptible subjects.

Third, more detailed genotyping of the *ADRB2* locus or haplotype analysis might have yielded additional insight into the relationship between genetic variations of *ADRB2*, but neither genome-wide association study or haplotype data were available for all cohorts. We focused on the SNP rs1042713 because there is a large body of related literature, which indicates that this is associated with outcomes for asthma treatment.

A fourth limitation is that we have assumed that treatment has been assigned based on the same criteria, and it is possible that in some cohorts, children with more severe asthma and at increased risk for exacerbations might not have received LABA treatment, but this would tend to underestimate the effect of the interaction between LABA treatment and the SNP rs1042713 for exacerbations.

A fifth limitation is that rare variants were not genotyped, which could also have an effect on adverse events during LABA therapy; however, these cohorts were not powered for a rare variant analysis, and the rare variants identified by Ortega et al[37] occurred on the background of Gly16 but not Arg16.

Finally, the Gly16Arg allele polymorphism frequency was not in HWE for the whole BREATHE cohort, but the consistency of the results across the cohorts suggests that the deviation within this single cohort did not substantially influence the overall results. Furthermore, Gly16Arg is an inconsistently replicated and, at best, weak locus for asthma severity, and therefore it does not seem plausible that selection for exacerbation (the primary outcome and linked to asthma severity) in this study is the cause for the deviation from HWE seen in the BREATHE cohort. Because Gly16Arg is a better established pharmacogenetic locus, a more plausible outcome that would influence deviations from HWE is exacerbations or symptoms despite frequent SABA or LABA use, but this represents a small percentage of the BREATHE cohort (18% and 11%, respectively).

In conclusion, children with asthma receiving an ICS plus LABA were at 52% increased risk for exacerbation in the previous 6 to 12 months for each A allele (Arg16 amino acid) compared with G/G homozygotes (Gly16/Gly16). Given that there are 1 million children in the United Kingdom with asthma, 10% of these are prescribed LABAs,[38] and 60% of these carry at least 1 Arg16 allele, there are approximately 60,000 children in the United Kingdom today and approximately 25,000 in The Netherlands who might be at risk from the morbidity of exacerbation, which is preventable by treatment with either no LABAs or perhaps with the addition of an LTRA if genotyping of rs1042713 could be made available at the point of prescribing. Put another way, and assuming that at least one third of the UK national health care costs attributable to childhood asthma are due to urgent care,[39,40] stratified treatment might reduce the annual direct costs to the United Kingdom National Health Service for the management of childhood asthma exacerbations by 10% (ie, a 50% reduction in 60% of the population). Although there are no recent published costs for the management of childhood asthma, costs in the United States have been estimated at $791 per child per annum in 2005[40] and to total £150 million in the United Kingdom in 1997.[41] Thus it is likely that stratified treatment in childhood asthma will save tens of millions of pounds in direct health care costs. Not all asthmatic patients who inherit an A allele will experience increased morbidity with LABA treatment, and this might be explained by rare variants, perhaps at the same locus[37]; pathway-related variation[42]; epigenetic mechanisms; or treatment adherence.

Key message

- **Clinical trials are required to determine whether treatment stratified by rs1042713 will reduce asthma exacerbation risk in children with 1 or 2 A alleles.**

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METHODS

BREATHE study

The BREATHE study is cohort of children and young adults with doctor-diagnosed asthma recruited from primary and secondary care in Tayside. Details of enrollment have been presented in detail previously.[E1] Subjects aged more than 18 years were excluded from the present analysis.

GALA II study

The Genes-Environment and Admixture in Latino Americans (GALA II) study is a multicenter study of children and young adults with and without asthma and has been fully described previously.[E2] Eligibility criteria were as follows: age of 8 to 21 years, all 4 grandparents were Latino, and smoking history was less than 10 pack years. Asthma was defined based on a physician's diagnosis and report of symptoms and medication use within the last 2 years. Subjects aged more than 18 years and without asthma were excluded from this analysis. Estimates of African, European, and Native American ancestries were obtained by using an unsupervised analysis in ADMIXTURE,[E3] assuming 3 ancestral populations: African, European, and Native American. We used reference haplotypes from subjects from HapMap phase II (http://hapmap.ncbi.nlm.nih.gov) for the European and African components: Utah residents with Northern and Western European ancestry from the CEPH collection (CEU) and Yoruba in Ibadan, Nigeria (YRI). The Native American reference population consisted of 71 Native American subjects genotyped at the University of California San Francisco on the Axiom LAT1 array, including 14 Zapotec, 2 Mixe, and 11 Mixtec from the southern State of Oaxaca and 44 Nahua subjects from Central Mexico.

PAGES

PAGES (http://www.asthma-pages.com/) was designed to explore interactions between genetic variations and exposures (including medications) in children with asthma. Children were recruited from primary and secondary care. Details of recruitment have been published previously.[E4] Ethnicity was not identified for the initial recruits to the study but was captured for approximately half participants and categorized as African, Chinese, Indian, Mixed, Pakistani, White British, or other.

PACMAN cohort

PACMAN is a study of children aged 4 to 12 years recruited through community pharmacies in The Netherlands between 2009 and 2012. Details of the study protocol have been described elsewhere.[E5] A detailed history of the subjects was obtained, including information on asthma symptoms, exacerbations, and medication use over the preceding 12 months during a study visit in the community pharmacies. Ethnicity was categorized as African, Asian, white (Dutch, Turkish, or Moroccan), Hispanic, or mixed.

PASS

PASS is a cohort study designed to explore the clinical and pharmacogenomics associations between use of corticosteroids in children with asthma and adrenal suppression. Assessment included a respiratory questionnaire and collection of blood or salvia for DNA extraction. Details of recruitment have been published previously.[E6] Children with Asian ancestry were excluded, and ethnicity for those recruited was categorized as African, white, or other.

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TABLE E1. Power of the sample size of the 5 cohorts combined to detect increases in ORs for asthma exacerbations

Treatment group	OR = 1.2	OR = 1.5	OR = 3
SABA alone	44%	96%	100%
ICS	70%	99%	100%
ICS+LABA	36%	91%	100%
ICS+LTRA	20%	64%	100%
ICS+LABA+LTRA	34%	89%	100%

TABLE E2. OR for exacerbation regardless of treatment in each cohort from the additive model

	BREATHE	GALA II	PACMAN	PAGES	PASS	Pooled
OR (95% CI)	1.14 (0.97-1.34), n = 1210	1.18 (0.98-1.41), n = 1171	1.00 (0.69-1.41), n = 760	1.02 (0.82-1.28), n = 695	1.08 (0.81-1.43), n = 390	1.11 (1.01-1.22)

The OR indicates the risk for each A allele of rs1042713. The logistic regression models adjusted for age, sex, and exposure to cigarette smoke. The *P* value for the pooled analysis was .035.

TABLE E3. OR and 95% CI for exacerbation for each Arg16 allele compared with Gly 16 homozygotes among children receiving a daily SABA regardless of other treatment

	BREATHE	PACMAN	PAGES	Pooled
OR (95% CI) for children in receipt of SABA every day	1.13 (0.77-1.65), n = 250	1.41 (0.83-2.40), n = 364	0.69 (0.44-1.10), n = 208	1.01 (0.79-1.31)

The logistic regression models adjusted for sex, age, and exposure to cigarette smoke. The *P* value for the pooled analysis was .91.

TABLE E4. OR and 95% CI for exacerbation by using the additive model (ie, risk for each Arg16 allele compared with none) among children treated with ICSs and LABAs for the 3 populations in which SABA use was determined

	BREATHE	PACMAN	PAGES	Pooled
OR (95% CI) for children in receipt of SABA every day	1.02 (0.37-2.81), n = 44	4.57 (1.40-14.89), n = 74	0.89 (0.30-2.63), n = 47	1.49 (0.79-2.79)
OR (95% CI) for children in receipt of SABA less than once a day	1.71 (0.94-3.12), n = 94	0.65 (0.06-19.51), n = 72	1.55 (0.80-2.99), n = 87	1.59 (1.03-2.45)

The logistic regression models adjusted for sex, age, and exposure to cigarette smoke. The *P* values for the pooled analysis were .22 and .038.